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Effects of the pyrethroid insecticide cypermethrin on a freshwater community studied under field conditions. II. Direct and indirect effects on the species composition

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Abstract

The effects of cypermethrin, a commonly used pyrethroid insecticide, were studied in small in situ enclosures situated in an eutrophic lake over an 11-day period. The experimental design used a regression principle that included three untreated controls and a gradient of six unreplicated cypermethrin concentrations, ranging from 0.01 to 6 μg/l. This paper is the second in a series of two and describes the effects on the species composition of the crustacean, rotifer, periphyton and phytoplankton communities. Multivariate ordination technique (redundancy analysis (RDA) combined with Monte Carlo permutation tests) showed that exposure to cypermethrin caused significant changes in the species composition of the communities. Changes in the structure of the communities were observed following exposure to a nominal concentration of 0.13 μg cypermethrin per litre above. The direct acute effect of exposure to cypermethrin was a rapid decrease of many species of crustacean zooplankton. The alterations in crustacean species composition were probably due to variations in susceptibility to the direct toxic effects of cypermethrin. No effects concentration (NEC) for individual zooplankton species were calculated using inverse regression and revealed that copepod *nauplii* were the most sensitive (NEC = 0.01 μg/l) of the crustacean groups examined. The observed alterations of the species composition of the autotrophic communities as well as of the rotifers were most likely caused indirectly by cypermethrin, mediated through the direct negative effects of the insecticide on the crustacean grazers. The results of this experiment provide further knowledge about the direct and indirect effects of pesticide stress on the ecosystem level. They also show that there is a variation in sensitivity between different species of zooplankton under natural conditions and thus exemplify the necessity of multispecies approaches in the risk assessment of pesticides.

Keywords: Cypermethrin; Algae; Zooplankton; Freshwater; Ecosystem effect; Species composition; Indirect effects

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1. Introduction

The use of pesticides has increased rapidly since World War II, and several recent studies show that pesticide residues frequently occur in surface water in agricultural areas (Høysæter, 1994; Larson et al., 1999; Ulén et al., 2002). Many aquatic species are taxonomically related to the target organisms of pesticides. Aquatic ecosystems in agricultural areas are thus at risk of being negatively affected by these chemicals. Risk assessment of chemicals is still based on the effects on individuals, and data are often generated only through single species toxicity tests (van Leeuwen, 1995a,b; EU, 1997). However, the species used in such tests may not be representative of species occurring in natural waters and are unlikely to represent the most sensitive species (Cairns, 1986). The variation in sensitivity between different species can also be several orders of magnitude (Blanck et al., 1984; Källqvist and Romstad, 1994). Furthermore, organisms exposed to chemicals in their natural surroundings may be more (or less) sensitive to toxicants than organisms exposed in the laboratory, because of effects such as density dependence and stress induced by food shortage or competition. Neither do single species laboratory tests incorporate the alterations of ecosystems resulting from indirect effects of the pesticide. Hence it is difficult to extrapolate the effects resulting from single species laboratory studies to effects on natural ecosystems. Therefore, it is necessary to increase our knowledge about how aquatic ecosystems as a whole may be affected by pesticides. Studies in experimental ecosystems are important since these studies can improve our understanding of both direct and indirect processes involved in chemical stress of ecosystems and may provide knowledge for a priori prediction of effects on the ecosystem level.

The purpose of the present study was to examine the effects of cypermethrin, a pyrethroid insecticide, on a plankton communities in an eutrophic freshwater ecosystem. This paper is the second in a series of two and describes the direct and indirect effects on the species composition within different trophic levels. The variation in sensitivity between different species of zooplankton is examined, and the similarities and divergences in sensitivity compared with laboratory studies are discussed. The potential different mechanism by which exposure to insecticides may indirectly alter the species composition of communities not directly affected are also discussed. The first paper focuses on the effects of exposure to cypermethrin on the abundance and biomass of organisms at different trophic levels (Friberg-Jensen et al., 2002).

2. Materials and methods

2.1. Experimental design

The experiment was conducted in nine pelagic polyethylene enclosures (diameter: 44 cm; depth: 150 cm) filled with 200 l of surface water from the eutrophic Lake Fredriksborgs Slotssø, Denmark in June, 1999.

Six of the enclosures ($E1-E6$) were treated with cypermethrin (Riedel-deHaen, 96% cypermethrin, analytical standard grade) on day 0, while the remaining three enclosures served as controls ($C1-C3$). The treatment concentrations were prepared by dilution of a stock solution of cypermethrin in acetone and subsequently mixed into the water column by gentle stirring, resulting in nominal concentrations of 0.01, 0.04, 0.13, 0.47, 1.7 and 6.1 μg cypermethrin per litre. Further details of the experimental set-up can be found in Friberg-Jensen et al. (2002).

2.2. Planktonic communities

Depth-integrated water samples were taken using a plastic tube (width: 3.5 cm; length;120 cm) from five positions evenly distributed within each of the enclosures.

For the identification and counting of crustaceans and rotifers, a one-litre sub-sample was filtered through a 200 μm mesh net (mesozooplankton) and subsequently through a 50 μm mesh net (rotifers and *nauplii*) 4 and 12 h after application of the pesticide and after 0, 1, 2, 4, 7 and 11 days. The retained animals were transferred to clean bottles and fixed with Lugol's solution.

Counting and identification to species or genus level was carried out using an inverted microscope (Olympus IMT-2, 100–400 × magnification). Subsamples for the determination of the phytoplankton species composition were taken on days 0, 7 and 11 and preserved with Lugol's solution. Phytoplankton were identified to species or genus level and counted in an inverted microscope (Nikon Diaphot TMD, 200–400 × magnification) using sedimentation chambers (25 ml). The biovolume was calculated using measurements of the cells in the microscope and geometrical formulas describing the shape of the cell (Olrik et al., 1998).

2.3. Periphyton

Periphyton was sampled from unglazed ceramic tiles (3.7 cm^2) that served as artificial substrates. Before the experiment the tiles were allowed to be precolonised by a periphytic community for 10 days in the eutrophic Lake Bysjön (Scania, Sweden, Coveney et al., 1977). At the start of the experiment (day 0), the tiles were positioned 10 cm below the water surface in each of the enclosures. At the end of the experiment (day 11) five tiles were gently brushed visually clean into 100 ml of tap water and preserved with 35% formalin. The periphytic algae taxa were identified to a genus level and counted in an inverted microscope (Nikon Diaphot TMD, 200–400 × magnification) using sedimentation chambers (25 ml). In addition loose heterocysts of *Anabena* sp. were frequently found and counted. Both filamentous and pseudoparenchymatous forms of *Stigeoclonium* sp. occurred in the periphyton. Possibly, both forms belong to the same species, but their biomasses were estimated separately. The biovolume was calculated using measurements of the cells in the microscope and geometrical formulas describing the shape of the cell (Olrik et al., 1998).

2.4. Data analysis

Multivariate ordination techniques were used to create an overall picture of the effects of exposure to cypermethrin on the species composition of the zooplankton, periphyton and phytoplankton communities by means of the software CANOCO, version 4 (Ter Braak and Smilauer, 1998). The hypothetical gradient, obtained using detrended correspondence analysis (DCA), driving the variation in the species composition was <1.5 S.D., therefore, linear ordination techniques, i.e. Principal component analysis (PCA) and redundancy analysis (RDA), were used (Van Wijngaarden et al., 1995). The biomass values of the phytoplankton and periphyton species and the numbers of zooplankton were $\ln(A \times X+1)$-transformed (where X is the abundance value for the species, and $A = 2/(\text{lowest abundance values})$) before analysis as recommended by Van den Brink et al. (2000). PCA was used to obtain a graphical summary of the variation in the species composition of the samples. In PCA, hypothetical environmental variables that best explain the variation in the species data are calculated. The eigenvalues of the axis are a measure of the importance of the axis in explaining the variance in the data set and in this paper are expressed as the fraction of variance in the species data accounted for by each axis (Van Wijngaarden et al., 1995). The ordination diagrams (Figs. 1, 3, 5 and 7) are distance biplots resulting from PCA. The following description of the interpretation of these plots is based on Ter Braak (1994) and Ter Braak and Smilauer (1998). Samples with similar species composition are close together. A ranking of the fitted abundance of a particular species in the samples can be obtained by projecting the sample points perpendicularly onto the line resulting from an extension of the species arrow. The highest fitted abundance of the species can then be found in the sample which projects furthest away in the direction in which the species arrow points, the second highest fitted abundance can be found in the sample projecting second furthest away, and so forth. The direction in which a species arrow points is the direction in which the species abundance value increases the most. The length of a species arrow is a measure of the rate of change of the species fitted abundance along the axis.

In order to test whether the variation in the species data was significantly related to the environmental variable, RDA was used in combination with a Monte Carlo permutation test (Ter Braak

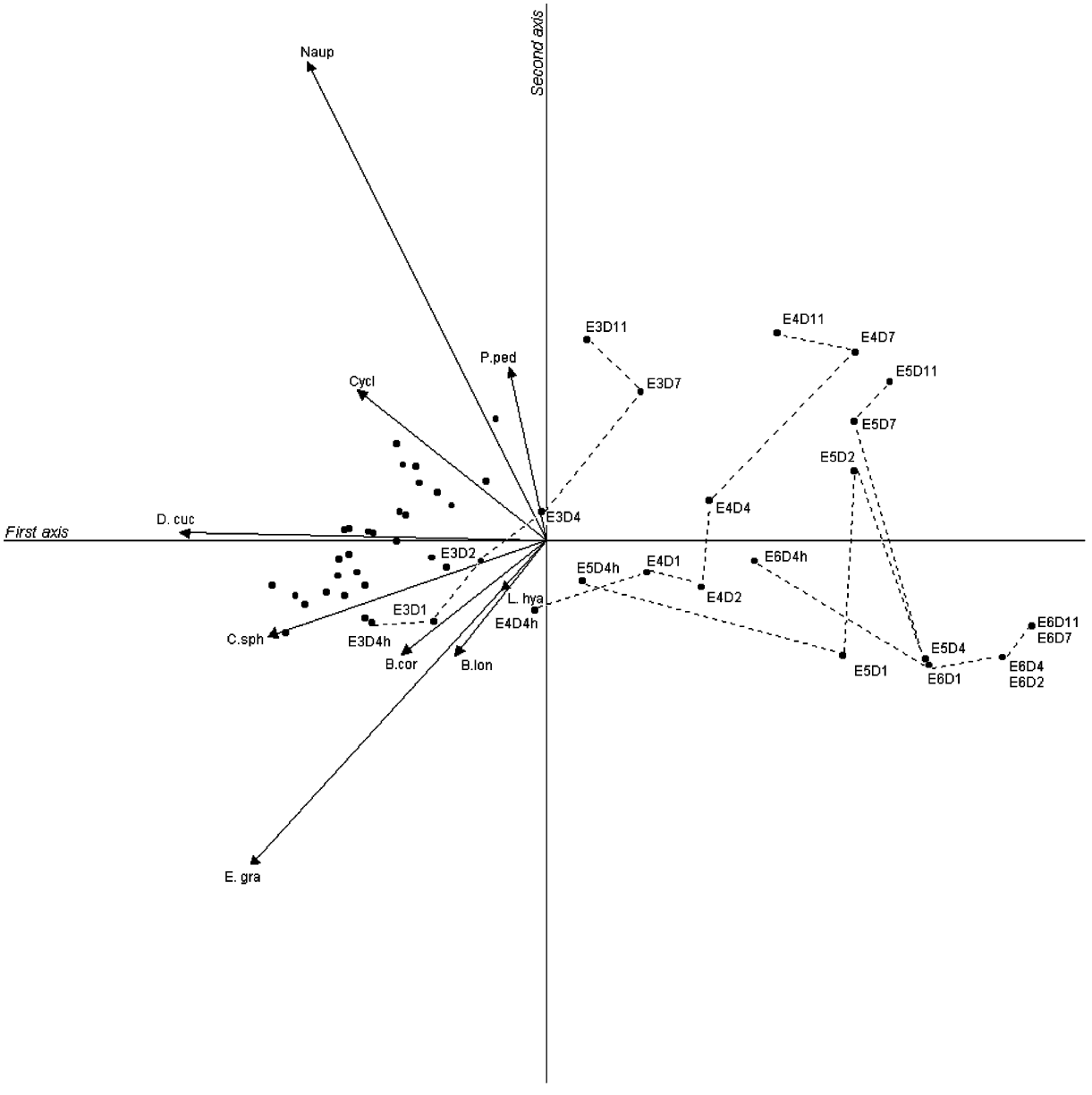

Fig. 1. Distance biplot based on the PCA of the crustacean data. Species with less fit than 10% are not shown in the diagram. Eigenvalues of the first three axes are 0.73, 0.10 and 0.06. The lines indicate the course of the alterations caused by the treatment in enclosures $E3$–$E6$. In order to avoid overcrowding the diagram, the sample labels are only given for enclosure $E3$–$E6$. X:4 h refers to samples taken 4 h after the exposure, X:1 refers to samples from day 1 and so forth. D. cuc, *Daphnia cucullata*; B. lon, *Bosmina longirostris*; B. cor, *B. coregoni*; C. sph, *Chydorus sphæricus*; L. hya, *Leptodora hyalina*; P. ped, *Polyphemus pediculus*; E. gra, *Eudiaptomus graciloides*; Cyc, *Cyclops*; Naup, *Nauplii*. The nominal exposure concentrations of the enclosures were $C1$–$C3 = 0$, $E1 = 0.01$, $E2 = 0.04$, $E3 = 0.13$, $E4 = 0.47$, $E5 = 1.7$ and $E6 = 6.1$ μg cypermethrin per litre.

and Smilauer, 1998). RDA is a constrained form of PCA in which the explanatory axes are constrained to be linear combinations of the environmental variables (Jongman et al., 1987; Ter Braak and Smilauer, 1998). The Monte Carlo permutation tests were performed for each sampling date using the nominal concentration of cypermethrin as the explanatory variable. The treatment concentrations were ln-transformed before testing. The level of significance was set to be 0.05. For a more substantial background of the general concepts of RDA analysis combined with Monte Carlo permutations, see Ter Braak and Smilauer (1998).

The no effect concentration (NEC) for the different zooplankton species was calculated according to an inverse regression procedure based on the actual estimated cypermethrin concentrations, as described in more detail in Friberg-Jensen et al. (2002).

The effect of the treatment on the occurrence of loose *Anabena* sp. heterocysts in the periphyton was analysed by means of simple linear regression. We chose to analyse the occurrence of the heterocysts separately, as they were an unexpected extra observation which we do not consider belongs to the 'true' algal community. The number of hererocysts was log-transformed and the nominal cypermethrin concentrations were $\log(100 \times X+1)$-transformed in order to meet the requirements of homogenity of variance and normalised statistical distributions

3. Results

3.1. Zooplankton

The control crustacean community was dominated by the cladoceran *Daphnia cucullata*, the calanoid copepod *Eudiaptomus graciloides* and copepod *nauplii* (including those from cyclopoids). Other frequently encountered taxa were the cladoceran *Chydorus sphæricus* and cyclopoid copepods *Cyclops* spp. Carnivorous cladocerans (*Leptodora hyalina* and *Polyphemus pediculus*) were present but not abundant. All the control sample points and the sample points from the enclosures exposed to 0.01 and 0.04 μg cypermethrin per litre ($E1$ and $E2$) are located close together in the ordination diagram (Fig. 1), indicating a similar species composition in these samples and small changes over time. The sample points from enclosures exposed to ≥ 0.13 μg/l ($E3$–$E6$) are found towards the right in the diagram (relative to controls) directly following treatment, indicating that the abundance of all cladoceran zooplankton decreased rapidly in these enclosures. On later sampling days the sample points from the enclosures exposed to 0.13–1.7 μg/l ($E3$–$E5$) moved towards the upper right-hand corner of the diagram, indicating a recovery of *nauplii* in these enclosures. The diagram also shows that the abundances of *E. gracilodies*, *Bosmina* spp., *C. sphæricus* and *D. cucullata* did not recover during the experiment. The RDA combined with Monte Carlo permutation test showed that exposure to cypermethrin had a significant effect on the crustacean species composition as early as 4 h after the addition ($P = 0.015$), and that the sum of the canonical eigenvalues, which is the percentage of the variance in the data set that can be explained by the exposure to cypermethrin, was 60%. Furthermore, the effect of cypermethrin exposure was significant on all subsequent sampling days ($P = 0.005$) and explained between 70 and 85% of the variation in the species data.

The calculation of NEC showed that *nauplii* were the most sensitive group directly following cypermethrin application. The NEC was 0.01 μg cypermethrin per litre and LC_{50} was 0.05 μg cypermethrin per l 4 h after the application (Table 1), calculating using the actual estimated concentration of cypermethrin (see Friberg-Jensen et al., 2002). For reasons discussed in Friberg-Jensen et al. (2002) it was not possible to calculate *nauplii* NEC-values on subsequent sampling days. In general, *Cyclops* spp. was the least sensitive taxon (Table 1) with a NEC of 0.09 μg cypermethrin land and a LC_{50} of 0.18 g cypermethrin per litre 24 h after the application of cypermethrin. This can also be seen in the graphs describing the responses of individual zooplankton taxa (Fig. 2) which

Table 1
NEC (μg/l) and effect concentrations (EC_{50}, μg/l) for crustacean taxa based on actual estimated cypermethrin concentrations

	Sample time	NEC (μg/l)	LC50 (μg/l)	*n*	r^2
E. graciloides	4 h	0.13 (0.010–0.338)	0.27 (0.042–0.886)	4	0.94
	Day 1	0.04 (0.030–0.059)	0.07 (0.046–0.131)	3	0.99
	Day 4	0.01 (<0.001–0.058)	0.03 (0.002–0.186)	5	0.94
Cyclops spp.	4 h	0.04 (<0.001–0.471)	0.17 (0.001–*)	4	0.94
	Day 1	0.09 (<0.001–0.480)	0.18 (<0.001–2.563)	3	0.93
Nauplii	4 h	0.01 (<0.001–0.131)	0.05 (<0.001–*)	5	0.91
D. cucullata	Day 1	0.03 (0.001–0.172)	0.05 (0.002–*)	4	0.96
	Day 2	0.03 (0.007–*)	0.05 (0.009–*)	4	0.99
	Day 4	0.02 (0.006–0.082)	0.03 (0.008–*)	3	0.99
	Day 11	0.03 (0.02–0.057)	0.04 (0.02–*)	3	0.93

Numbers in brackets are 95% confidence limits. *n*, the number of data points included in the regression analysis (*n* max = 6), r^2, the coefficient of correlation for the regression line, *, missing value.

show that the abundance of *nauplii* decreased rapidly directly following exposure to 0.13 μg/l while the response of the other taxa were slower.

The dominating taxa in the rotifer control community were *Keratella* sp., followed by *Pompholyx* sp., *Kellicottia longispina* and *Asplanchna*

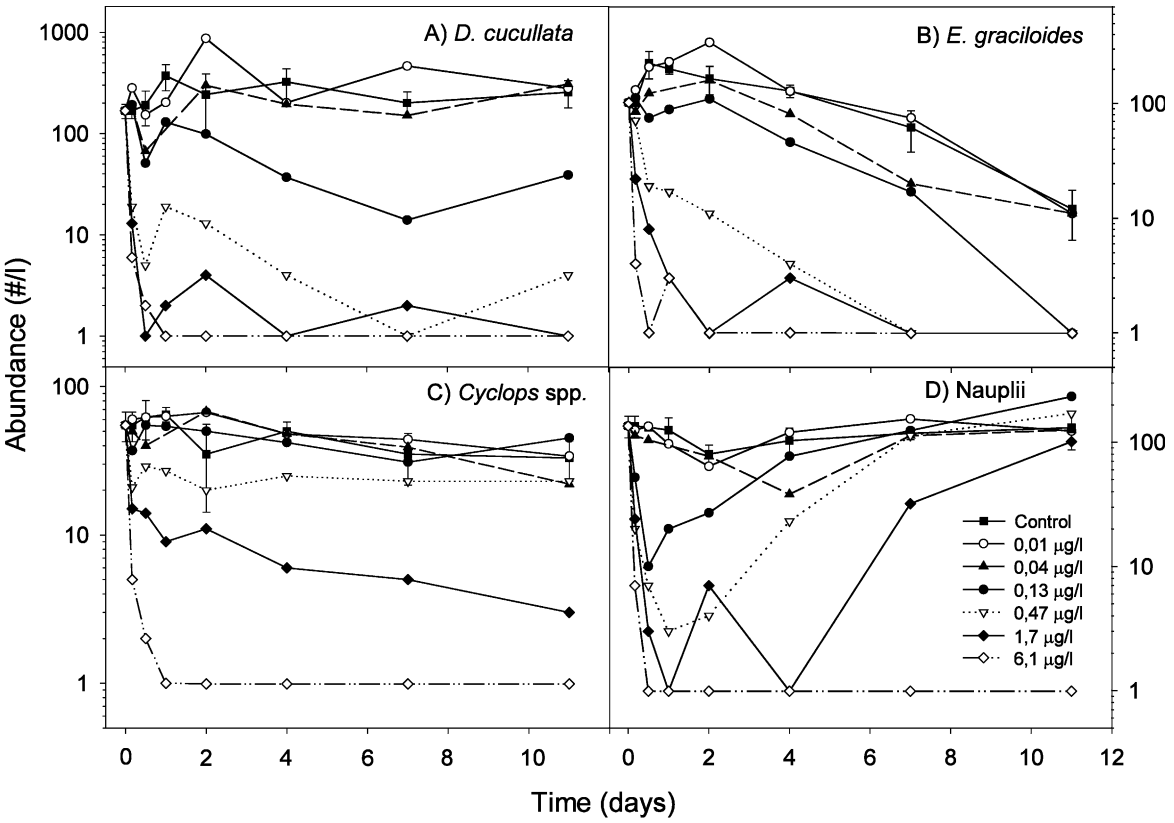

Fig. 2. The abundances of (A) *D. cucullata* (B) *E. graciloids* (C) *Cyclops* spp. (D) *Nauplii* in relation to the nominal concentration of cypermethrin.

sp. The sample points from the enclosures exposed to 0.13–6 μg/l (*E*3–*E*6) on day 11 are situated close together and to the far right in the PCA diagram indicating that the species composition in these enclosures were similar to each other as well as different from those of the other enclosures (Fig. 3). Furthermore, also the *E*3–*E*6 sample points from day 7 were situated to the right in the diagram. The RDA combined with a Monte Carlo permutation test showed that at the last sampling occasion a large proportion of the variance in the species composition of the rotifer community could be significantly explained by the cypermethrin treatment ($P = 0.005$, canonical eigenvalue 61%). Significant treatment effects on the species composition of the rotifer community were also found on day 1 and day 4 (RDA, $P = 0.05$). On these sampling occasions, however, only a small fraction ($< 22\%$) of the variation in the species data could be attributed to the treatment and these sample points do not cluster closely together or differently from the other points in the PCA biplot. The taxa most closely correlated to the first axis in the RDA and thus also to the treatment on day 11 were. *Filinia* sp., *K. quadrata*, *K. cochlearis* and *Polyarthra* sp. The percentage contribution of each species to the total abundance also changed in response to the exposure to cypermethrin (Fig. 4). The percentage contribution of *Lepadella*/*Euclanis* sp. was lower in enclosures exposed ≥ 0.04 μg/l (*E*2–*E*6) compared with controls on day 11. Similarly *Pompholyx* sp. and *K. longispina* had a low relative abundance in enclosures exposed to ≥ 0.47 μg/l, while the relative abundance of *K. quadrata* and *Filinia* sp. increased in response to treatment (Fig. 4).

3.2. Phytoplankton

The ordination diagram indicates that the species composition of the phytoplankton in the enclosures changed both over time and with treatment (Fig. 5), with the effects of time mainly displayed on the first axis and the effects of treatment on the second. The abundances of *Anabena* sp. and *Chrysochromulina parve* increased with increasing concentrations of cypermethrin, while the abundances of *Oocystis* sp., *Microcystis* sp. and *Cyanodictyon* sp. were highest in the control enclosures. The abundances of *Cyclotella* sp., and *Rhodomonas* sp. decreased over time, however, more so in the control and low exposure enclosures. Monte Carlo permutation tests showed that the variation in the taxa composition was significantly related to the nominal concentration of cypermethrin on days 7 and 11 ($P = 0.01$). The sum of the canonical eigenvalues was 62% on day 7 and 61% on day 11. A similar pattern was found for the changes in percentage contribution of each taxon to the total biomass (Fig. 6).

3.3. Periphyton

The sample data points from enclosures exposed to ≥ 0.13 μg cypermethrin per litre (*E*3–*E*6) are located to the left in the distance biplot (Fig. 7), which indicates that there were overall increases in the abundance of periphytic algae in these enclosures since all species arrows, except for *Nitzschia* sp., point to the left. However, the abundance of each species increased to a varying extent, as can be seen from the variation in the lengths of the species arrows, with *Cyclotella* sp., *Kirchneriella* sp., *Oedogonium* sp. and *Planktolyngbya* sp. increasing the most in response to treatment. This is also shown in Fig. 8 where it as can be seen that the percentage contributions of *Cyclotella* sp., *Oedogonium* sp. and *Planktolyngbya* sp. in the periphyton increased. Meanwhile the percentage contributions of *Phacotus* sp., the pseudoparenchymatous and filamentous form of *Stigeoclonium* sp., and *Nitizschia* sp. decreased. RDA combined with Monte Carlo permutation tests showed that the changes in periphytic species abundance were significantly related to the concentration of cypermethrin ($P = 0.01$; canonical eigenvalue = 51%).

Anabena sp. filaments were not found frequently in the periphyton and, therefore, not counted, however, heterocysts of *Anabena* sp. were abundant and increased significantly in response to cypermethrin treatment (simple linear regression, $P < 0.001$, $r^2 = 0.93$) (Fig. 9).

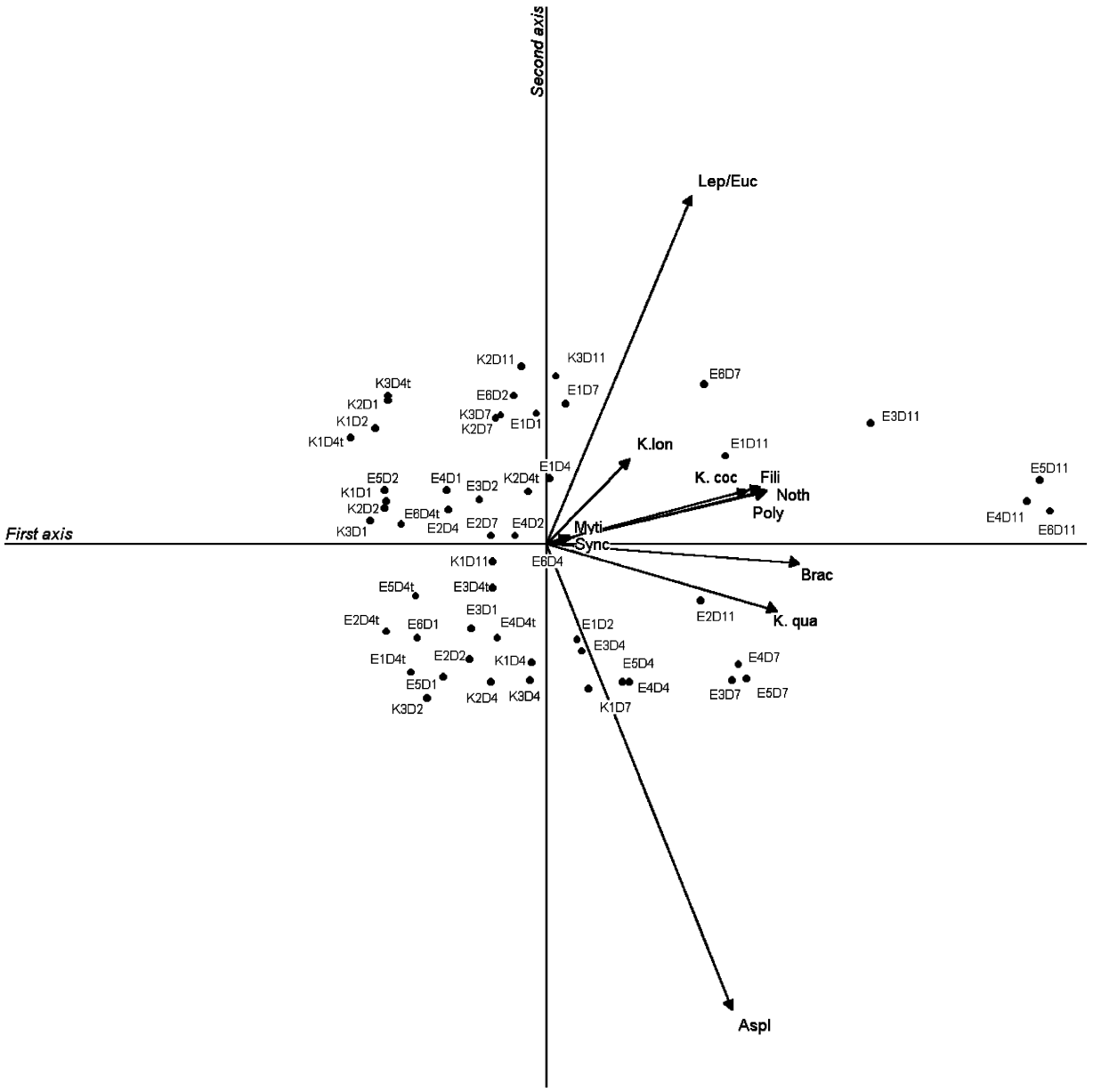

Fig. 3. Distance biplot based on the PCA of the rotifer data. Species with less fit than 10% are not shown in the diagram. Eigenvalues of the first three axes are 0.37, 0.15 and 0.10. X:4 h refers to samples taken 4 h after the exposure, X:1 refers to samples from day 1 and so forth. Aspl, *Asplanchna*; K. coc, *Keratella cochlearis*; K. qua, *Keratella quadrata*; K. lon, *Kellicottina longispina*; Brac, *Brachionus* sp.; Poly, *Polyarthra* sp.; Lep/Euc, *Lepadella* sp. and *Euclanis* sp.; Myti, *Mytilina* sp.; Fili, *Filinia* sp.; Noth, *Notholca* sp.; Sync, *Synchæta* sp. The nominal exposure concentrations of the enclosures were $C1-C3=0$, $E1=0.01$, $E2=0.04$, $E3=0.13$, $E4=0.47$, $E5=1.7$ and $E6=6.1$ μg cypermethrin per litre.

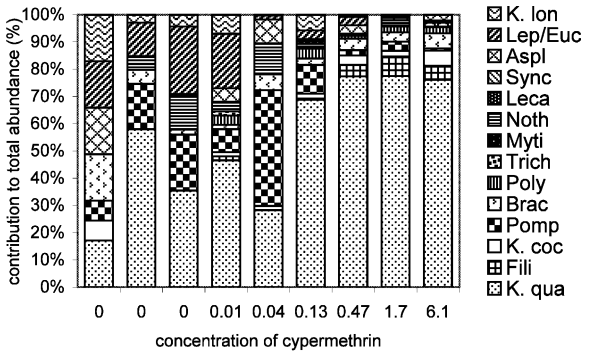

Fig. 4. The percentage contribution of each rotifer taxa to the total numbers on day 11 in relation to the nominal concentration of cypermethrin. Aspl, *Asplanchna*; K. coc, *Keratella cochlearis*; K. qua, *Keratella quadrata*; K. lon, *Kellicottina longispina*; Brac, *Brachionus* sp.; Poly, *Polyarthra* sp.; Lep/Euc, *Lepadella* sp. and *Euclanis* sp.; Myti, *Mytilina* sp.; Fili, *Filinia* sp.; Noth, *Notholca* sp.; Sync, *Synchæta* sp.; Tric, Tichocerca; Pomp, Pompholyx; Leca, *Lecane* sp.

4. Discussion

The direct effects of exposure to cypermethrin resulted in a drastic decrease of all crustaceans (Friberg-Jensen et al., 2002). However, the sensitivity varied between species resulting in an altered species composition as can be seen both in the ordination diagram (Fig. 6) and in Table 1 with NEC and EC_{50} values for the different species. In our study, the cyclopoid copepod was the least sensitive and *nauplii* the most sensitive zooplankton taxa examined (Fig. 2 Table 1). The *nauplii* decreased immediately after the exposure but increased again during the experiment at all except the highest concentration. The concentration of cypermethrin in the enclosures at the point when the recovery of *nauplii* started was around 0.03 μg/l, calculated using the data from the cypermethrin measurements assuming first order kinetics (Friberg-Jensen et al., 2003). The rapid recovery of *nauplii* in the exposed enclosures, might have been a result of an induced stress response causing surviving female copepods to increase egg production. In addition it may also have been the increased food availability (algae and other microorganisms, see Friberg-Jensen et al., 2002) for surviving females that resulted in an enhanced reproduction of copepods. Similar observations, i.e. an increased abundance of copepod *nauplii*, were made by Farmer et al. (1995) following application of lambda-cyhalothrin to artificial ponds, as well as by Wendt-Rasch et al., 2003 following exposure of freshwater pond enclosures to cypermethrin.

Laboratory-derived $L(E)C_{50}$ for *D. magna* exposed for 24 and 48 h is typically found to be in the range of 0.3–5 μg cypermethrin per litre (nominal as well as actual concentrations, Stephenson, 1982; Hill, 1985; Day, 1989; WHO, 1989; Kjølholt et al., 1991). Our field-derived EC_{50}-values for *D. cucullata* (Table 1) are clearly lower than the laboratory $L(E)C_{50}$ (24 and 48 h) for *D. magna* reported in the literature, and even up to two orders of magnitude lower than the higher $L(E)C_{50}$-values reported. Compared with laboratory-derived LC_{50} (96 h) for the adult marine calanoid copepod *Acartia tonsa* of 0.142 μg cypermethrin per litre (Medina et al., 2002), the field-generated EC_{50} for adult copepods in the present study is lower. On the other hand the laboratory-derived LC_{50} (96 h) of 0.005 μg/l for *nauplii* of *A. tonsa* (Medina et al., 2002) is lower than the present field-generated *nauplii* EC_{50}. However, the field *naplii* EC_{50} is calculated for the sampling 4 h after exposure and, most likely, EC_{50} values generated 12 and 24 h after the initial exposure would have resulted in a lower value (the reason for the lack of these values in the present study is discussed in Friberg-Jensen et al., 2002). In accordance with other studies which have indicated that neonate daphnids (Buhl et al., 1993; Day and Kaushik, 1987; Hanazato, 2001) and copepods (Medina et al., 2002) are more sensitive than the respective adult individuals to a range of pesticides, our study also revealed lower NEC- and EC_{50}-values for *nauplii* than for adult individuals of *Cyclops* spp. and *E. graciloides*. Higher sensitivity of juvenile than of adult crustaceans may be a result of a larger surface-volume ratio leading to a larger relative exposure compared with adult crustaceans, as well as a higher frequency of moulting in juveniles, since moulting and recently moulted individuals may be more sensitive to toxicant exposure (Buikema et al., 1980; Gliwicz and Sieniawska, 1986). The observed differences between our field-derived EC_{50} and laboratory results can be due to a differences

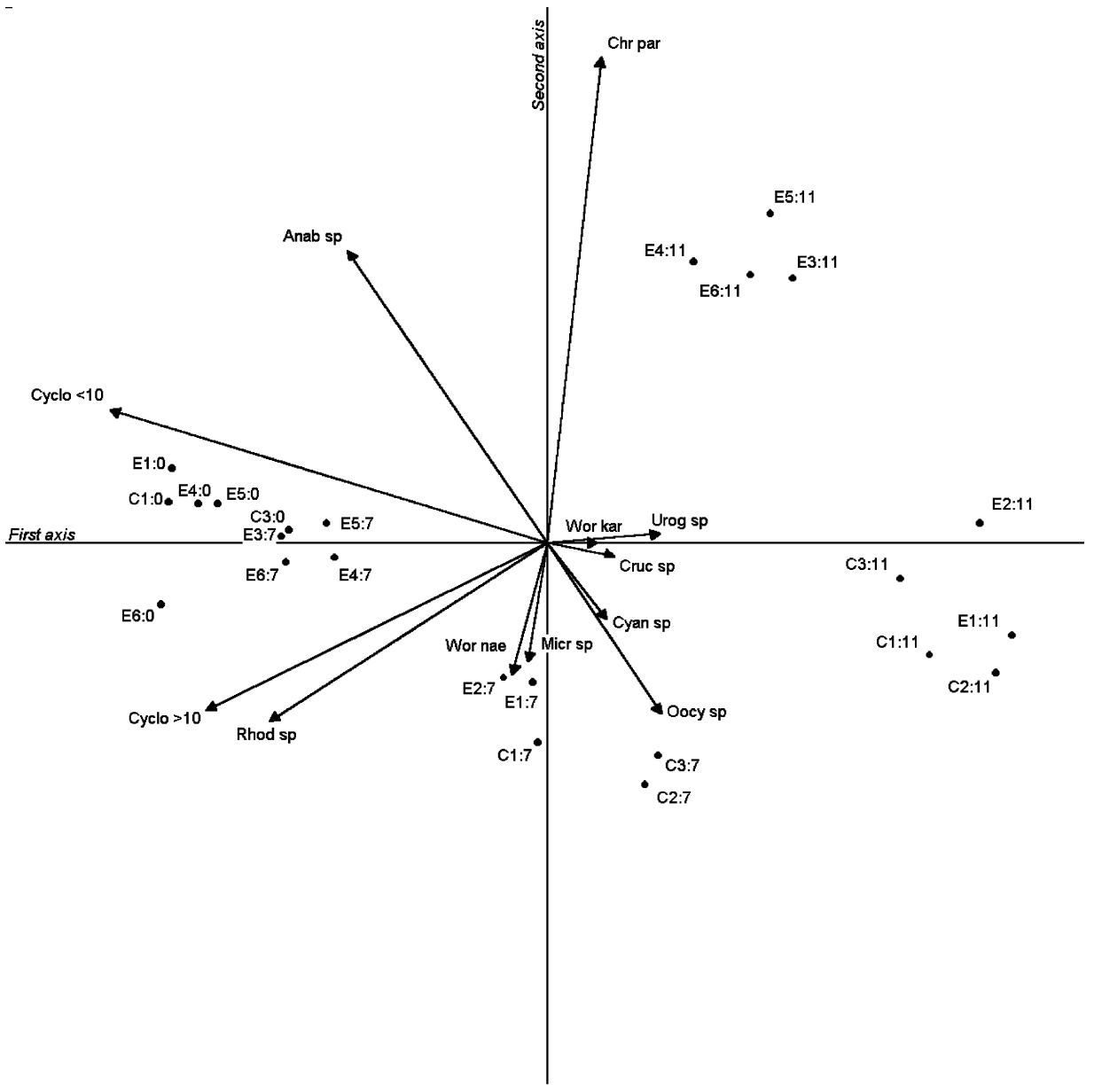

Fig. 5. Distance biplot based on the PCA of the phytoplankton data. Species with less fit than 10% are not shown in the diagram. Eigenvalues of the first three axes are 0.69 0.187 0.041. X:0 refers to preexposure samples, X:7 refers to samples from day 7 and X:11 refers to samples from day 11. Cyclo > 10 = *Cyclotella* sp. ≥ 10 μm, Cyclo < 10 = *Cyclotella* sp. ≤ 10 μm, Cruc sp., *Crucigenia* sp.; Urog sp., *Uroglena* sp.; Chr par, *Chrysochromulina parve;* Rhod sp., *Rhodomonas* sp.; Oocy sp., *Oocystis* sp.; Wor/Snow, *Woronichinia* sp. and *Snowella* sp., Micr sp., *Microcystis* sp.; Anab sp., *Anabena* sp.; Cyan sp., *Cyanodictyon* sp. The nominal exposure concentrations of the enclosures were $C1–C3 = 0$, $E1 = 0.01$, $E2 = 0.04$, $E3 = 0.13$, $E4 = 0.47$, $E5 = 1.7$ and $E6 = 6.1$ μg cypermethrin per litre.

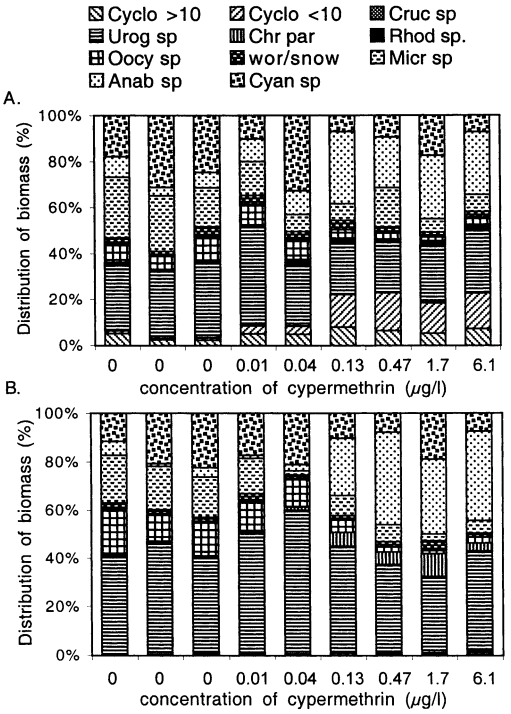

Fig. 6. (A) The percentage contribution of each phytoplankton taxa to the total biomass on day 7 in relation to the nominal concentration of cypermethrin. (B)The percentage contribution of each phytoplankton taxa to the total biomass on day 11 in relation to the nominal concentration of cypermethrin. Cyclo > 10 = *Cyclotella* sp. _ > 10 μm, Cyclo < 10 = *Cyclotella* sp. ≤ 10 μm, Cruc sp., *Crucigenia* sp.; Urog sp., *Uroglena* sp.; Chr par, *Chrysochromulina parve*; Rhod sp., *Rhodomonas* sp.; Oocy sp., *Oocystis* sp.; Wor/Snow, *Woronichinia* sp. and *Snowella* sp.; Micr sp., *Microcystis* sp.; Anab sp., *Anabena* sp.; Cyan sp., *Cyanodictyon* sp.

in the sensitivity between the species used in laboratory tests and the species studied in the present field study. Another fact that may have contributed to the observed discrepancy is that laboratory organisms are reared under optimal conditions which may result in populations that are more tolerant to toxicants compared with natural populations. The latter may be subjected to sub-optimal food concentrations and quality as well as to fluctuating temperature and even other toxic compounds such as cyanobacterial metabolites that may increase the organisms sensitivity. The observed variation in sensitivity between species in the present experiment emphasises the importance of testing several species as well as life stages in order to generate data for risk assessment of chemicals.

Tolerable or 'safe' concentrations of pesticides are often extrapolated from single species laboratory studies in the first tier of risk assessment, either using an extrapolation factor, or more recently using species sensitivity distributions (van Leeuwen, 1995a,b; EU, 1997). When the proposed safety factor of 100 for acute exposure (EU, 1997) is applied to the toxicity data for *D. magna*, cited above, the result is a suggested 'safe' concentration of 0.002–0.05 μg/l. In the probabilistic risk assessment of cotton pyrethroids conducted by Solomon et al. (2001), the species sensitivity distribution of cypermethrin acute toxicity data gave a tenth centile value of 0.0064 μg/l for aquatic arthropods, which the authors suggest as a 'safe' concentration for ecosystem protection. Often these extrapolation methods are validated by comparing the generated 'safe' concentrations with NOECs or NECs generated in field studies or multispecies tests. When the 'safe' concentrations, generated through the probabilistic method as well as using the safety factor approach using the lower reported LC_{50}-values for *D. magna* are compared with the NEC values for crustaceans in the present study, the extrapolated 'safe' concentrations appear to be protective of the community in the present field study. However, for reasons discussed in Friberg-Jensen et al. (2002) the confidence limits surrounding the NEC-values were broad in the present study, ranging between 0.001–0.48 μg/l, and the lower range of these values is actually lower than the 'safe' concentrations extrapolated from single species tests. It is, therefore, questionable whether field studies, such as the present, should be used to generate 'safe' concentrations or to validate 'safe' concentrations extrapolated from single species tests. If such comparisons are to be done it is extremely important that the often large variation in multispecies tests, and accordingly the often low power to detect significant effects, are considered and reported.

The toxicity of pyrethroid insecticides to freshwater algae is in general low (> 1000 μg/l; Hill, 1989), and thus the observed effects on the species composition of the periphytic and the planktonic

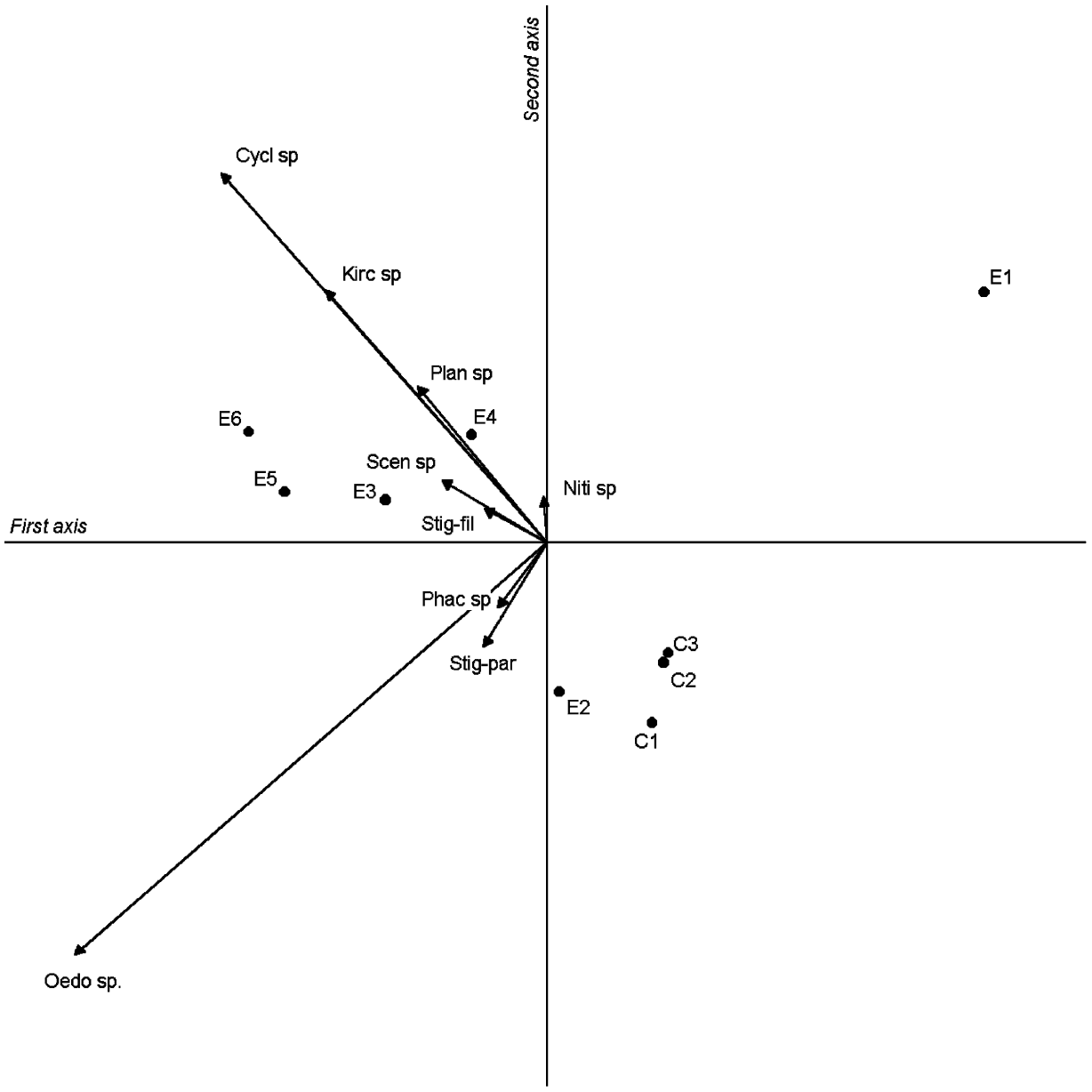

Fig. 7. Distance biplot based on the PCA of the periphyton data. Species with less fit than 10% are not shown in the diagram. Eigenvalues of the first three axes are 0.65, 0.27, 0.05. Cycl sp., *Cyclotella* sp.; Niti sp., *Nitizschia* sp., Kirc sp.; *Kirchneriella* sp.; Scen sp., *Scenedesmus* sp.; Stig-par, Pseudoparenchymateous form of *Stigeoclonium* sp.; Stig-fil, Filamentous form of *Stigeoclonium* sp.; Oedo sp., *Oedogonium* sp.; Phac sp., *Phacotus cf. Linearis*; Plan sp., *Planktolyngbya* sp. The nominal exposure concentrations of the enclosures were $C1-C3=0$, $E1=0.01$, $E2=0.04$, $E3=0.13$, $E4=0.47$, $E5=1.7$ and $E6=6.1$ μg cypermethrin per litre.

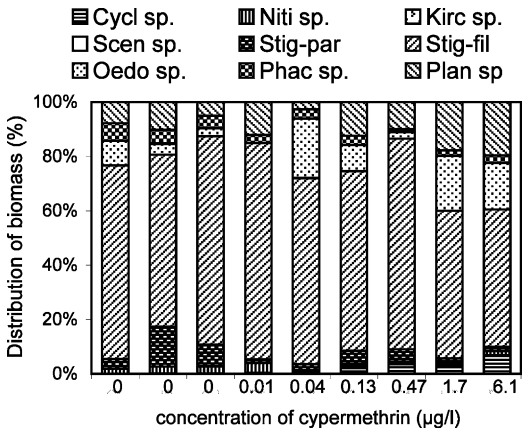

Fig. 8. The percentage contribution of each periphytic taxa to the total biomass in relation to the nominal concentration of cypermethrin. Cycl sp., *Cyclotella* sp.; Niti sp.; *Nitizschia* sp., Kirc sp., *Kirchneriella* sp.; Scen sp., *Scenedesmus* sp.; Stig-par, Pseudoparenchymateous form of *Stigeoclonium* sp.; Stig-fil, Filamentous form of *Stigeoclonium* sp.; Oedo sp., *Oedogonium* sp.; Phac sp., *Phacotus cf. Linearis*; Plan sp., *Planktolyngbya* sp.

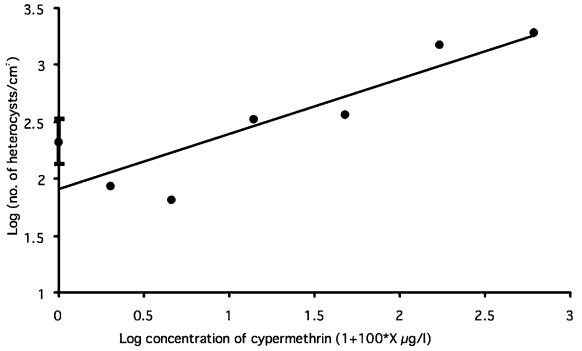

Fig. 9. The number of heterocysts/cm^2 (log-transformed) found on the ceramic tiles plotted vs. the nominal concentration of cypermethrin. The error bars refer to the standard deviation of the three control enclosures.

algae communities (Figs. 5–8) can probably be attributed to indirect effects of cypermethrin exposure. However, a combination of direct and indirect effects cannot be ruled out since the direct toxicity of cypermethrin and its metabolites to the algae species inhabiting our test systems has rarely been examined in single species tests. One possible indication of a direct effect of cypermethrin on the algae is the increased occurrence of loose *Anabena* sp. heterocysts in the periphyton which might have been a stress response to the cypermethrin exposure, although we cannot find any support for this speculation in the literature.

The abundance of the diatom *Cyclotella* sp., both sessile (on tiles) and planktonic was highest in the enclosures exposed to high concentrations of cypermethrin (Figs. 5–8). However, the abundance of the planktonic form of this genus also decreased over time making this difference less pronounced at the last sampling occasion. Other investigators have indicated that cladocerans are effective grazers on similar centric diatoms (Vanni, 1987; Pérez-Martínez and Cruz-Pizarro, 1995), and a decrease of cladocerans would therefore, similar to our observations, tend to favour the growth of *Cyclotella* sp. Moreover, the abundances of *Chrysochromulina parve* in the phytoplankton and *Kirchneriella* sp. in the periphyton increased in response to cypermethrin exposure. These species are within the edible size (< 15 µm) for zooplankton, and may thus have benefited from a decreased grazing pressure in the exposed enclosures. Further, the abundance of *Oocystis* sp., a species with gelatinous sheets in the plankton community, and the proportion of *Phacotus* sp., a species with thick cell walls in the periphytic community, were highest in the control and low exposure enclosures. Durable cell walls and gelatinous sheets may reduce the vulnerability of algae to grazing (Porter, 1973, 1975) allowing such species to predominate when the grazing pressure is high, as in the control enclosures. Accordingly, *Oocystis* sp. has been shown to decrease in enclosure experiments as a response to the addition of fish, which reduces the zooplankton algal grazing (Vanni, 1987; Pérez-Martínez and Cruz-Pizarro, 1995). This suggests that a decrease in the abundance of *Oocystis* sp. and *Phacotus* sp. may be due to a competitive disadvantage in environments with low grazing pressure, as prevalent in the enclosures exposed to cypermethrin.

However, it is not only the resistance or vulnerability to grazing that may determine the indirect effects of exposure to insecticides on the algal species composition. The variation in response of algae species to abiotic factors that can be influenced by changes in the zooplankton community, such as nutrient concentration and ratios,

may also alter the species composition. Zooplankton have been shown to strongly modify nutrient ratios through differential nutrient recycling (Elser et al., 1988; Sommer, 1988) and nutrient cycling by zooplankton can be an important source of nutrients for phytoplankton (Lehman, 1980; Carpenter et al., 1992). A manipulation study showed that phytoplankton were predominantly nitrogen-limited when grazed by small zooplankton and phosphorus-limited when grazed by large zooplankton (Elser et al., 1988). A low nitrogen to phosphorus ratio has been suggested to favour the growth of nitrogen fixing cyanobacteria (Smith, 1983). Thus, it is possible that the insecticide induced shift of the zooplankton community towards dominance by rotifers (Friberg-Jensen et al., 2002) decreased the $N{:}P$ ratio, which may explain the increase of *Anabena* sp. (a heterocystous genus) in the plankton community (Fig. 9). However, Jensen et al. (1994) did not find any correlation between the occurrence of heterocystous cyanobacteria and nitrogen availability. Instead, they found that heterocystous cyanobacteria dominated at low phosphorus concentrations, non-heterocystous at intermediate, while chlorophytes were dominant at high phosphorus concentrations in Danish lakes. Thus, an additional explanation may be that the decreased zooplankton grazing resulted in an overall decrease in the recycling and, consequently, in the availability of phosphorus favouring the heterocystous cyanobacteria *Anabena* sp. Fredriksborg Slotssø, where the present study were performed, is a highly eutrophic lake (Christoffersen et al., 1993) and it might therefore, seem unlikely that the phytoplankton could be nutrient limited. However, the experiment was performed during the clear water phase, when the lake also has a thermocline, and algal growth might actually have been dependent on recycled nutrients. Furthermore, there was an indication of an overall increase in the proportion of cyanobacteria in the exposed enclosures on day 11, with 50–55% of the total algal biomass being cyanobacteria in enclosures $E4$–$E6$ while the cyanobacterial contribution in the controls was between 40 and 45% (Fig. 5). This is in accordance with other studies (e.g. Schoenberg and Carlson, 1984; Christoffersen et al., 1993; Sarnelle, 1993) which have shown that daphnid grazing reduces the proportion of cyanobacteria in the phytoplankton community.

There was a significant increase in the abundance of rotifers in the exposed enclosures towards the end of the experiment (Friberg-Jensen et al., 2002). However, the different species increased to a varying extent, resulting in an altered relative distribution of species (Figs. 3 and 4). This was probably a result of the decreased abundance of cladocerans and especially *Daphnia*, which effectively controlled the rotifer population in the control enclosures. The ability of *Daphnia* spp. to control the abundance of rotifers is often explained by the effects of interference competition and/or exploitative competition for the same food source (Gilbert, 1988). Competition from *Daphnia* sp. and other crustaceans will most likely affect different rotifer species differently and is therefore, one of the factors structuring the species composition of the rotifer community. In the enclosures exposed to cypermethrin the major alterations of the rotifer species composition were an increased contribution of *K. quadrata* and *Filinia* sp., while *K. longispina*, *Lepadella*/*Euclanis* and *Pompholyx* sp. decreased (Fig. 4). *K. cochlearis* has been found to be very sensitive to inference competition (Gilbert, 1988) and thus the observed increase of similar sized species of the same genera in the present study might accordingly have been a response to the decreased daphnia inference competition in the exposed enclosures. A similar effect was also found in a study by Day et al. (1987) in which *Keratella* spp. was found to be largely responsible for the increase in rotifers which followed the exposure to fenvalerate, another pyrethroid insecticide. We could not find any general pattern regarding the influence of the size on the response to the treatment. Studies have indicated that small rotifer species in general are more sensitive to inference competition than large species (Gilbert, 1988). Thus, the decreased abundance of daphnids in the exposed enclosures would tend to favour small rotifer species. However, *Daphnia* and other cladocerans may also suppress rotifer abundance through exploitative competition for food. Large rotifer species are probably more susceptible than small species to food

limitation (Gilbert, 1988; Sarma et al., 1996). The increased food availability (e.g. increased algal biomass, bacteria, ciliates and heterotrophic flagellates) in the cypermethrin-exposed enclosures (Friberg-Jensen et al., 2002) would hence, unlike the decreased inference competition, tend to favour large-bodied species. Thus, the decreased competition from cladocerans caused by exposure may thus favour both large and small species, through different mechanisms, which might explain the absence of a pattern.

In the present experiment both direct and indirect effects were observed following a single application of a nominal concentration of 0.13 μg cypermethrin per litre and above. In the risk assessment procedure adopted by the European Union it is recommended that predicted environmental concentrations (PECs) should be calculated on the basis of a 30 cm deep body of water and appropriate spray drift percentages (European Commission, 2001). Using the labelled application rate for cypermethrin (25–150 g a.i./ha) in such calculations, the 0.13 μg/l-treatment corresponds to an approximate 0.2–1.5% drift. In a single application a drift of 2.8% (90 percentile) may contaminate water bodies situated at a distance of 1 m from treated fields (European Commission, 2001). Hence, the present study indicates that negative effects on aquatic ecosystems adjacent to agricultural land cannot be excluded following normal agricultural use of cypermethrin.

In conclusion, the results from this study clearly emphasise the importance of ecosystem level tests when assessing the effects of pesticides on aquatic ecosystems in order to fully understand the processes involved in pesticide stress on aquatic ecosystems. However, the usefulness of this type of multi-species test in generating 'safe' concentrations for ecosystem exposure may be limited due to the very broad confidence limits surrounding the NECs and EC_{50}s. This experiment shows that direct effects of exposure to cypermethrin on the crustacean zooplankton community propagate to the planktonic and periphytic algal, bacteria, protozoan and rotifer communities both in terms of effects on the abundance (see Friberg-Jensen et al., 2003) and community structure (present paper). The effects resemble alterations caused by eutrophication, and thus the exposure of aquatic ecosystems to insecticides may add to the adverse effects caused by an increased nutrient load.

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